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Distance1D – a protein profile analytical program designed for fungal taxonomy

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Abstract —Taxonomic analysis of macromycete fruiting bodies is a challenging task that utilizes morphological, biochemical, and molecular methods. Many biochemical and molecular methods have been developed to test or confirm identifications or phylogenetic positions independently of morphological data. SDS electrophoresis has been shown to be a good biochemical method for protein separation. Although protein profiles can be analyzed by commercially available software, there is no software designed specifically for fungal taxonomic research. We have developed an open source portable program that uses protein profiles of fungal fruiting bodies to calculate relative differences between species for use in generating to generate more accurate phylogenetic trees.

Key words — biochemistry, fungi, Perl, proteome

Introduction

The challenging task of macromycete taxonomy uses morphological, biochemical, ecological, and molecular data (Guarro et al. 1999, Blackwell et al. 2006, Korabecna 2007, Lutzoni & Vilgalys 1995). Sodium dodecyl sulfate (SDS) electrophoresis is a good biochemical method for protein separation and biomarker discovery, which when applied to the proteomes of fungal fruiting bodies gives a good number of separated proteins, revealing small (or sometimes large) differences in protein expression (Materić 2012). The method, which is robust and reliable, could serve as an independent taxonomic tool for fungi (Guarro et al. 1999, Tyrrell 1969).

The aim of this work was to develop a user-friendly open source program using electrophoresis data for fungal taxonomy. Such a program would compare and calculate relative differences between taxa based on results from SDS gel electrophoresis and similar techniques (e.g., western blot, zymography).

Materials & methods

We sampled and identified 21 fruiting bodies representing differently related species. Proteins were separated according to the method described in Materić (2012), and protein profiles were obtained for each species. The quality of protein profiles depends upon many factors during sample homogenization, protein extraction and electrophoresis, and an optimized protocol can be found in Materić et al. (2012). In this work we have chosen to show the results of only seven species: however, more detailed dendrograms are available in Materić (2012).

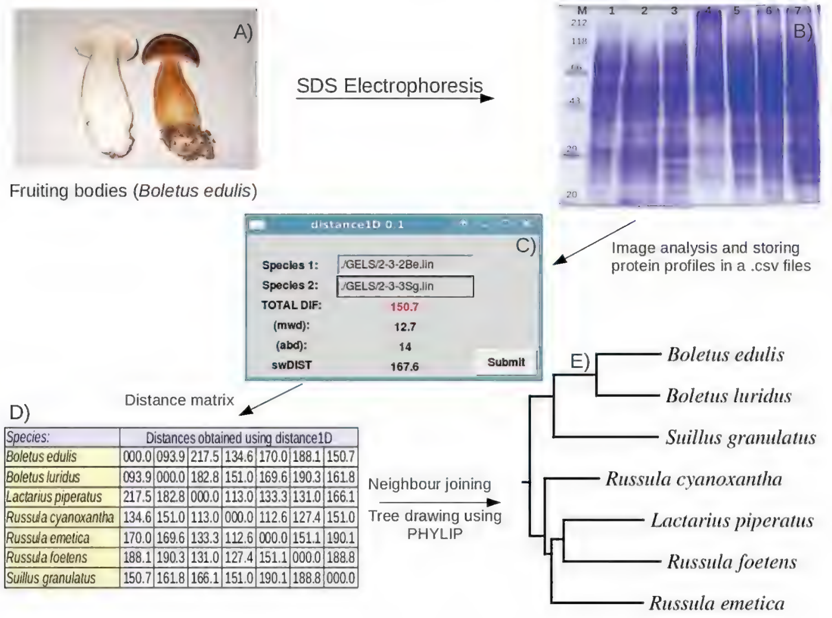
Protein profile data are stored in .csv format and examples of files can be found along with the source-code in Materić (2013). Protein profile files include two sets of data: (1) molecular weights (MW) of proteins; and (2) relative abundance. MW and relative abundances can be obtained using specialized software such as TotalLab (Phoretix, Newcastle, United Kingdom). However, abundances should be recalculated as relative abundances with range of 1 (least abundant) to 5 (most abundant). In order to create a distance matrix table, protein profiles are compared by distance1D. The distance matrix tables are transformed into dendrograms using the program package PHYLIP (Felsenstein 2002).

Results & discussion

Protein electrophoresis, which has been widely used in solving taxonomic and systematic problems, produces results that are independent of morphological and molecular data (Guarro et al. 1999, Tyrrell 1969). Thus, it is crucial to have appropriate software that deals with taxonomic issues rather than with pure biochemical properties. Apart from distance1D, there is no such software specifically designed to interpret fungal taxonomy from protein profile data.

Distance1D is a program designed to calculate relative differences between two protein profiles, which could then be used to generate dendrograms (Fig. 1). The program is designed and written specifically for use in fungal taxonomy (mainly for analyzing protein profiles of fungal fruiting bodies), but its usage could be wider. The program is meant to be open source, user-friendly, and portable. Portability results from the program's being written in Perl, which can be run on any operating system. As the program uses the Tk library for generating widgets (Windows-like interface), Perl/Tk should be installed along with Perl.

Our previous work produced good protein profiles of fungal fruiting bodies from SDS gel electrophoresis (Materić 2012) that are stored in separate files (Fig. 1).



Species 1: /GELS/2-3-2Be.lin
Species 2: /GELS/2-3-3Sg.lin
TOTAL DIF: 150.7
(mwd): 12.7
(abd): 14
swDIST 167.6

Species:	Distances obtained using distance1D						
Boletus edulis	000.0	093.9	217.5	134.6	170.0	188.1	150.7
Boletus luridus	093.9	000.0	182.8	151.0	169.6	190.3	161.8
Lactarius piperatus	217.5	182.8	000.0	113.0	133.3	131.0	166.1
Russula cyanoxantha	134.6	151.0	113.0	000.0	112.6	127.4	151.0
Russula emetica	170.0	169.6	133.3	112.6	000.0	151.1	190.1
Russula foetens	188.1	190.3	131.0	127.4	151.1	000.0	188.8
Suillus granulatus	150.7	161.8	166.1	151.0	190.1	188.8	000.0

FIGURE 1. Diagram showing stages of an experiment used in order to gain biochemically based phylogenetic trees. A: Fruiting bodies. B: Result of SDS electrophoresis (channels: M = molecular mass marker; 1 = *Boletus luridus*; 2 = *B. edulis*; 3 = *Suillus granulatus*; 4 = *Russula foetens*; 5 = *Lactarius piperatus*; 6 = *R. cyanoxantha*; 7 = *R. emetica*; Materić et al 2012). C: Interface of the program distance1D. D: Distance matrix table generated by the program. E: Phylogenetic tree drawn by PHYLIP program package (similar results were obtained by analyzing ITS II regions of rDNA as control; Materić 2012).

The program as an input takes the paths of two files (two file names) where protein data are stored in .csv format. An algorithm searches for optimal pairing of proteins by looping through the parameters, such as MW sensitivity. After alignment and optimization, the following differences are scored: (1) presence of new protein lines (new protein), (2) molecular weight difference of protein pairs, and (3) abundance difference of protein pairs. The scoring system, which could be adapted according to user requirements, is set up as follows: "10" scored for each new protein line, "1" scored for each 1kDa MW shift, and "2" scored for abundance difference. If DNA/RNA gel data are going to be used, the abundance difference for each fragment should be set the same.

There are several important advantages to using this program: the program generates distances that give accurate phylogenetic trees (more accurate than a single DNA sequence such as rDNA ITS1; Materić 2012); the parameters can be changed to suit particular research; the program can be easily adapted for use with any protein/DNA/RNA electrophoresis data; and the program is suitable for all operating systems and is open source and free.

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